Published in final edited form as:
*Cancer*. 2009 April 1; 115(7): 1498–1506. doi:10.1002/cncr.24157.

# *Cyclooxygenase-2* Gene Polymorphisms Reduce Risk of Oral Premalignant Lesions

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## Abstract

Oral premalignant lesions (OPLs) have the potential to transform into malignant oral cancers. The overexpression of *cyclooxygenase-2* (*COX-2*) gene is frequently found in OPLs and oral cancers, suggesting that this gene may play an important role in the progression of oral cancer. Single nucleotide polymorphisms (SNPs) of *COX-2* gene have been associated with the risk of multiple cancers, but their effects on OPL susceptibility have not been sufficiently evaluated. Here we conducted a case-control study including 147 patients with OPL and 147 healthy matched controls. We evaluated the effects of three potentially functional *COX-2* polymorphisms, including −765G>C (rs20417), exon10+837T>C (rs5275), and exon10−90C>T (rs689470), on OPL risk. We found that the variant-containing genotypes of *COX-2* exon10+837T>C variant were associated with a significantly reduced OPL risk with an odds ratio (OR) of 0.48 (95% CI, 0.28−0.80). This protective effect was also significant in males, younger subjects, ever smokers, and ever drinkers. Consistently, a common haplotype (WMW, in the order of −765G>C, exon10+837T>C, and exon10−90C>T; W, wild-type allele, M, variant allele) and a common diplotype (WWW/WMW) that contained the variant allele of exon10+837T>C were both associated with a reduced OPL risk, having ORs of 0.55 (95% CI, 0.33−0.93) and 0.44 (95% CI, 0.22−0.89), respectively. In addition, using never smokers with the variant-containing genotypes as the reference group, we observed an interaction effects between specific *COX-2* variants and tobacco smoking in the modulation of OPL risk. Overall, our results provided the first epidemiological evidence indicating that potentially functional polymorphisms of the *COX-2* gene may impact OPL susceptibility.

### Keywords

*COX-2*; polymorphism; haplotype; diplotype; oral premalignant lesion

## INTRODUCTION

Oral cancer accounts for 5% and 2% of all new cancer cases annually among males and females, respectively (1). The majority of oral cancer patients were diagnosed with advanced-stage disease, mostly due to the nonspecific clinical symptoms, especially in the

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young patients without traditional risk factors (2), thus highlighting the necessity of identifying clinically relevant susceptibility biomarkers for early detection and targeted chemoprevention. Oral premalignant lesions (OPLs), including mainly leukoplakia and erythroplakia, are important risk factors for oral cancer (3). The rate at which OPLs transform into oral malignancies varies between 11% and 36%, depending upon the geographic location of the individual and length of follow-up time (4). Both genetic and environmental factors contribute to OPL development. Epidemiological studies have identified the environmental risk factors, such as tobacco smoking, tobacco chewing, and alcohol drinking (5,6). However, the genetic components responsible for OPL formation are largely unknown.

*Cyclooxygenase-2* (*COX-2*) plays an important role in oral cancer development through catalyzing the biogenesis of inflammation-promoting prostaglandins. Chronic inflammation may induce neoplasia through the increased production of reactive oxygen and nitrogen species, which result in elevated DNA damage (7). Chronic inflammation may also induce the expression of multiple tumor-promoting genes (such as *TNF*, *MMP*s, and *VEGF)* that contribute to enhanced cellular migration and angiogenesis through the regulation of the proinflammatory gene nuclear factor-κB (7-9). *COX-2* has been consistently reported to be overexpressed in multiple malignancies and precursor lesions, including oral cancers and OPLs (1,10,11). Moreover, selective inhibitors of *COX-2* enzymatic activity have shown promising therapeutic potential in the treatment of oral cancers through the inhibition of multiple *COX-2* induced oncogenic pathways (10,12-14).

There have been a few studies evaluating the effects of SNPs on the predisposition of OPL. Our group and others have suggested that genetic polymorphisms in the angiotensin-converting enzyme gene *ACE*, cytochrome P450 gene *CYP1A1*, cell cycle progression gene *CCND1*, and nucleotide excision DNA repair genes were associated with OPL risk (15-18). SNPs of *COX-2* gene have also been associated with the etiology of a wide variety of solid tumors including oral squamous cell carcinoma (19-21). We recently found that the exon10+837, a potentially functional SNP located in the 3' untranslated region (UTR) of *COX-2* gene, may modulate the risk of bladder cancer through regulating the steady state mRNA expression of *COX-2* (22). Moreover, Lin et al. reported that a *COX-2* promoter variant, −765G>C, exhibited distinct effects on the development of different subtypes of oral cancer and OPLs (20). However, to our knowledge there have not been any studies assessing the associations of multiple functional variants of the *COX-2* gene with OPL susceptibility. In this study, we conducted a case-control analysis to evaluate the individual and haplotype/diplotype effects of the three most-studied potentially functional polymorphisms of *COX-2* gene on OPL predisposition. We also determined the modulating effects of specific patient characteristics as well as tobacco and alcohol exposures on the risk associations of these variations.

## MATERIALS AND METHODS

### Study subjects

The study included 147 OPL patients who were identified at The University of Texas M. D. Anderson Cancer Center between 1997 and 2006. The cases were histologically confirmed by the findings of OPLs (leukoplakia and/or erythroplakia). We excluded the patients younger than 18 years old and those with acute intercurrent illnesses or infections. Patients who had been treated for cancer within the preceding two years and those who received carotenoid or retinoid therapy within three months before the study also were excluded. Epidemiological data was gathered by using a self-administered questionnaire. Healthy controls (n = 147) were identified from a database of controls recruited in collaboration with the Kelsey-Seybold Clinic at Houston, consisting of over 300 physicians in 23 clinics.

Controls that had no history of cancer with the exception of non-melanoma skin cancer were matched strictly by age (± 5 years), gender, and ethnicity (Caucasian, African-American, and Hispanic) to the cases. This strategy for control recruitment has been well-described and has proven feasible and effective for molecular epidemiological studies when population-based control selection has posed a practical challenge (23). After the in person interview to obtain the epidemiologic questionnaire data, 40 ml blood sample was collected from each participant into a heparinized tube and sent to the laboratory for immediate molecular analysis. All the participants gave their written informed consent. The institution review board of both Kelsey-Seybold Clinic and M. D. Anderson Cancer Center have approved for the use of human subjects in this study.

## Genotyping

Three potentially functional SNPs of the *COX-2* gene were genotyped in this study, including one promoter SNP (−765G>C, rs20417) and two SNPs in 3' UTR (exon10+837T>C (rs5275) and exon10−90C>T (rs689470)). Genomic DNA was extracted from peripheral blood lymphocytes. Genotyping was conducted using 5' nuclease-based *TaqMan* real-time polymerase chain reaction (PCR) (Applied Biosystems, Foster city, CA). The primer and probe sequences were obtained from the National Cancer Institute's SNP500 Cancer database. The probes were labeled with FAM or VIC fluorescent dyes on the 5'end and a nonfluorescent minor groove binder quencher on the 3' end (Applied Biosystems, Foster City, CA). Typically, the PCR amplification mixes (5 μL) contained 5 ng of sample DNA, 200 μM of deoxynucleotide triphosphates, 1 × *TaqMan* buffer A, 5 mM of $MgCl_2$, 0.65 units of AmpliTaq Gold, 900 nM of each primer, and 200 nM of each probe. The thermal cycles consisted of 1 cycle for 10 minutes at 95°C, 40 cycles for 15 seconds at 95°C and 1 minute at 60°C. The ABI Prism 7900HT Sequence Detection System was used to read the reacted plates and SDS version 2.1 software (Applied Biosystems) was used to analyze the endpoint fluorescence. To ensure the accuracy of genotyping, water controls, internal controls, and previously genotyped samples were included in each plate. Laboratory personnel conducting genotyping were blinded to case-control status.

## Statistical analysis

The Stata 8.0 statistical software package (Stata Corp, College Station, TX) was used to conduct all the statistical analysis. The chi-square test was used to test for differences in the distribution of sex, smoking status, and ethnicity between cases and controls. The differences in the distribution of age and pack-years (among ever smokers) were assessed using student's *t*-test. An individual who had never smoked or had smoked less than 100 cigarettes in his or her lifetime was defined as never somker and a person who has smoked at least 100 cigarettes was defined as ever smoker. Individuals who never consumed alcohol or had no more than one drink per month were defined as never drinkers, and those who had more than one drink per month were defined as ever drinkers (one bottle or can of beer, one medium glass of wine, one straight shot or one mixed drink was defined as one drink). Hardy–Weinberg equilibrium (HWE) was analyzed using the goodness-of-fit chi-square test. The OPL risks were estimated as odds ratios (ORs) and 95% confidence intervals (CIs) using multivariate logistic regression analyses adjusted for age, gender, ethnicity, smoking status, and alcohol consumption. Haplotypes and diplotypes were estimated using the Expectation-Maximization algorithm implemented in HelixTree software (Golden Helix Inc, Bozeman, MT). Haplotypes with a probability of less than 95% were excluded from further analysis. The adjusted OR and 95% CI for each haplotype were assessed using multivariate logistic regression analysis adjusted for age, gender, ethnicity, smoking status, and alcohol consumption. Joint effect with tobacco smoking was assessed for each *COX-2* SNP using never smokers with the variant-containing genotype as the reference group. Interaction

between *COX-2* SNPs and smoking was analyzed by multivariate logistic regression model. All statistical tests were two-sided and the threshold of significance was 0.05.

## RESULTS

### Characteristics of the study population

The final study population consisted of 147 OPL patients and 147 healthy controls. No significant case-control differences were identified with regard to age (cases versus controls [mean ± standard deviation]: 57.48 ± 13.61 years versus 59.10 ± 11.04 years, $P = 0.26$) and gender ($P = 1.00$). As expected, ever smokers were statistically significantly overrepresented among the cases (68.71%) compared with controls (44.90%; $P < 0.001$). In addition, controls had a higher percentage of ever drinkers (68.03%) than did cases (53.74%) ($P = 0.012$).

### Individual *COX-2* Polymorphisms and the Risk of Oral Premalignant Lesions

In individual SNP analysis, after adjusting for age, gender, ethnicity, smoking status, and alcohol consumption, the minor allele of exon10+837T>C was associated with a significantly reduced OPL risk. Compared with individuals who had the homozygous wild-type genotype (WW), individuals with the heterozygous genotype (WM) and the homozygous variant genotype (MM) exhibited a reduced OPL risk with an adjusted OR of 0.49 (95% CI, 0.28−0.84; $P = 0.010$) and 0.44 (95% CI, 0.19−1.03; $P = 0.058$), respectively ($P$ for trend = 0.010, data not shown). The combined variant-containing genotypes (WW +WM) were associated with an OR of 0.48 (95% CI, 0.28−0.80; $P = 0.005$). In stratified analyses, this reduced risk remained significant in males (OR = 0.37; 95% CI, 0.17−0.78; $P = 0.009$), younger individuals (OR = 0.27; 95% CI, 0.12−0.61; $P = 0.002$), ever smokers (OR = 0.34; 95% CI, 0.16−0.71; $P = 0.004$), and ever drinkers (OR = 0.42; 95% CI, 0.21−0.84; $P = 0.013$) (Table 1). The −765G>C and exon10−90C>T SNPs did not show significance in the main analysis. However, the variant-containing genotype of −765G>C was associated with reduced risk (OR = 0.39; 95% CI, 0.18−0.84; $P = 0.016$) in ever smokers.

### Associations of *COX-2* Haplotypes With the Risk of Oral Premalignant Lesions

The relative risks associated with the common haplotypes of *COX-2* are shown in Table 2. Four common haplotypes GTC (WWW), GCC (WMW), CCC (MMW), and CCT (MMM) (in the order of −765G>C, exon10+837T>C, exon10−90C>T) were identified with a frequency of more than 1% in our study population. The most common haplotype WWW in which all alleles are wild-type occurred in 60% of the cases and 72% of the controls. Using this haplotype as a reference, the haplotype WMW, which contained the exon10+837T>C variant allele, was associated with a significantly reduced risk of OPL (OR = 0.55; 95% CI, 0.33−0.93; $P = 0.027$). This protective effect remained evident in females (OR = 0.42; 95% CI, 0.18−0.99; $P = 0.047$). Moreover, the haplotype MMW was associated with a significantly reduced risk in younger individuals (OR = 0.33; 95% CI, 0.13−0.83; $P = 0.019$), males (OR = 0.44; 95% CI, 0.20−0.96; $P = 0.040$), and ever-smokers (OR = 0.37; 95% CI, 0.17−0.81; $P = 0.013$).

### Associations of *COX-2* Diplotypes With the Risk of Oral Premalignant Lesions

Table 3 lists the common diplotypes of *COX-2* and their associations with OPL risk. Compared with the most common diplotype (WWW/WWW), the diplotype WWW/WMW was associated with a significantly decreased risk of OPLs, with an OR of 0.44 (95% CI, 0.22−0.89; $P = 0.022$). The diplotype WWW/MMW was associated with a borderline-significantly decreased risk with an OR of 0.51 (95% CI, 0.26−1.02; $P = 0.055$).

### Joint and Interaction Effects Between *COX-2* Genotypes and Tobacco Smoking

We found joint effects between all 3 *COX-2* SNPs and tobacco smoking. Using never smokers with the variant-containing genotypes as the reference group, we observed that the OR for individuals with wild-type alleles who were ever smokers was 2.99 (95% CI, 1.43−6.23) for the −765G>C SNP, 4.95 (95% CI, 2.48−9.91) for the exon 10 +837T>C SNP, and 15.60 (95% CI, 1.91−127.63) for the exon 10 −90C>T SNP (Table 4). In addition, we observed a statistically significant interaction effect between the −765G>C SNP and tobacco smoking (P for interaction =0.041) (Table 4).

## DISCUSSION

OPL is an important risk factor for oral cancer, thus, identification of individuals susceptible to development of OPL is critical for oral cancer prevention. In the current study, we assessed the individual and haplotype/diplotype effects of three potentially functional polymorphisms in the *COX-2* gene on OPL predisposition. We found that the variant allele of the exon10+837T>C SNP exhibited a significant protective effect against OPL risk, which was further confirmed in *COX-2* haplotype and diplotype analyses.

*COX-2* is an essential enzyme in the biogenesis of inflammation-promoting prostaglandins. Overexpression of *COX-2* has been identified in many solid tumors and premalignant lesions, including oral cancers and OPLs (1,10,11,24). The exon10+837T>C SNP is located in the 3’ UTR region, which may play an important role in the regulation of mRNA stability and translation. In our study, this SNP was found associated with a significantly reduced risk of OPL. We have previously shown that the variant allele of this SNP was also associated with a decreased risk of bladder cancer (22). In that study, we further demonstrated that the variant allele might affect cancer susceptibility through influences on the steady-state mRNA level of *COX-2* gene (22). In both studies, we found that the protective effect of this SNP remained significant in ever smokers, suggesting the presence of a potential joint effect between this SNP and tobacco-smoking. Furthermore, the predicted mRNA secondary structure analysis indicated an interruption of a 25 bp stem by the exon10+837T>C nucleotide change, leading to the production of a loop structure that may have an effect on mRNA stability (25). Consistent with our results, other researchers have related this SNP to a reduction in lung cancer risk in Korean and Chinese populations (26,27). However, this variant allele has also been associated with an increase in susceptibility to breast cancer in Austrians and lung cancer in Norwegians (25,27). Despite the differences in study design, sample size, and population stratification, the complicated role of *COX-2* in multiple inflammation-related malignancies might partially explain the apparent discrepancies in these findings. Larger studies are needed to provide independent epidemiologic validations and functional characterizations to confirm these findings.

In the current study, we found that the −765G>C SNP of *COX-2* was associated with a significantly reduced risk of OPL in heavy smokers. The promoter region of *COX-2* contains various transcriptional regulatory elements. Genetic variants in this region may change binding sites for transcriptional factors, thus altering the mRNA expression level. It has been reported that the −765G>C polymorphism, which is located in the 5’ to a STAT1 binding site, plays a role in carcinogenesis by abolishing a binding site of Sp1, a transcriptional activator of *COX-2* expression (28). Other studies have reported that this SNP might create an E2F binding site which regulates the expression of several important oncogenes or tumor suppressor genes (21,28-30). In line with our results, compared with the G allele of this polymorphism, the C allele was associated with a significantly lower level of promoter activity as well as reduced plasma C-reactive protein levels, and was implicated in many inflammatory responses (21,28). In further concordance with our results, the *COX-2* −765G>C polymorphism has also been identified to be associated with a reduced risk in

multiple malignancies including colorectal cancer (31), gastric cancer (30), non–small cell lung cancer (32), and bladder cancer (22). However, there are also a few studies indicating that the variant allele of this SNP conferred an increased cancer risk (20,33). These contradictions may be due to ethnic differences, study design, sample size, population heterogeneity, and cancer type-specific functions of the *COX-2* gene. Additional larger studies are needed to further validate our findings.

Haplotype and diplotype analyses indicated both the WMW haplotype and WWW/WMW diplotype that contained one copy of the variant allele of the exon10+837T>C SNP were associated with a significant protective effect on OPL (Tables 2 and 3). Moreover, both the MMW and MMM haplotypes that harbored this variant allele exhibited a nonsignificant protective effect. These findings were consistent with the conclusion provided by individual SNP analysis that the variant allele of *COX-2* exon10+837T>C conferred a reduced OPL risk. The MMW haplotype did not reach statistical significance in the main effect analysis, but was associated with a significantly altered risk in young subjects, males, and heavy smokers, indicating potential interactions between *COX-2* genotypes and specific patient characteristics.

Overall, we used strict matching criteria to eliminate the potential confounding effects of age and gender. We also have information on reported smoking status and alcohol consumption for each subject. We found that the *COX-2* exon10+837T>C polymorphism was associated with an altered risk of OPL. We also observed potential joint and interaction effects between specific *COX-2* SNPs and tobacco smoking. However, due to the relatively small sample size, we cannot rule out the possibility of chance findings in some of our results. Further studies are warranted to validate these results and investigate the underlying molecular mechanisms.

## Acknowledgments

Supported by National Cancer Institute grants CA 106451 and CA 097007.

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**Table 1**

Association of *COX-2* SNPs with OPL Risk Stratified by Patient Characteristics

| Variables | | *COX-2* −765G>C, rs20417 | | *COX-2* exon 10 +837T>C, rs5275 | | *COX-2* exon 10 −90C>T, rs68479 | |
|---|---|---|---|---|---|---|---|
| | | Case/Control | OR (95% CI)* | Case/Control | OR (95% CI) | Case/Control | OR (95% CI) |
| | | | *P* | | *P* | | *P* |
| **Overall** | | | | | | | |
| | WW** | 97/90 | Reference | 70/50 | Reference | 131/132 | Reference |
| | WM+MM** | 37/54 | 0.7 (0.41−1.20) | 66/95 | **0.48 (0.28−0.80)** | 5/13 | 0.43 (0.14−1.33) |
| | | | 0.197 | | **0.005** | | 0.143 |
| **Age** | | | | | | | |
| *Young* | WW | 48/41 | Reference | 37/21 | Reference | 65/62 | Reference |
| | WM+MM | 17/30 | 0.45 (0.19−1.05) | 31/49 | **0.27 (0.12−0.61)** | 3/9 | 0.34 (0.08−1.41) |
| | | | 0.063 | | **0.002** | | 0.136 |
| *Old* | WW | 49/49 | Reference | 33/29 | Reference | 66/70 | Reference |
| | WM+MM | 20/24 | 0.79 (0.36−1.75) | 35/46 | 0.68 (0.33−1.41) | 2/4 | 0.49 (0.07−3.42) |
| | | | 0.567 | | 0.301 | | 0.473 |
| **Gender** | | | | | | | |
| *Male* | WW | 56/45 | Reference | 37/24 | Reference | 74/73 | Reference |
| | WM+MM | 21/37 | 0.51 (0.24−1.07) | 41/57 | **0.37 (0.17−0.78)** | 4/9 | 0.43 (0.11−1.68) |
| | | | 0.076 | | **0.009** | | 0.223 |
| *Female* | WW | 41/45 | Reference | 33/26 | Reference | 57/59 | Reference |
| | WM+MM | 16/17 | 1.00 (0.42−2.35) | 25/38 | 0.47 (0.21−1.04) | 1/4 | 0.37 (0.04−3.53) |
| | | | 0.998 | | 0.062 | | 0.385 |
| **Smoking status** | | | | | | | |
| *Never* | WW | 26/51 | Reference | 19/26 | Reference | 43/70 | Reference |
| | WM+MM | 17/28 | 1.21 (0.55−2.66) | 25/54 | 0.65 (0.30−1.42) | 1/10 | 0.14 (0.2−1.17) |
| | | | 0.642 | | 0.282 | | 0.070 |
| *Ever* | WW | 71/39 | Reference | 51/24 | Reference | 88/62 | Reference |
| | WM+MM | 20/26 | **0.39 (0.18−0.84)** | 41/41 | **0.34 (0.16−0.71)** | 4/3 | 0.96 (0.18−5.03) |
| | | | **0.016** | | **0.004** | | 0.959 |
| **Alcohol consumption** | | | | | | | |

| Variables | | *COX-2* −765G>C, rs20417 | | *COX-2* exon 10 +837T>C, rs5275 | | *COX-2* exon 10 −90C>T, rs68479 | |
|---|---|---|---|---|---|---|---|
| | | Case/Control | OR (95% CI)* | Case/Control | OR (95% CI) | Case/Control | OR (95% CI) |
| | | | *P* | | *P* | | *P* |
| *Never* | WW | 44/32 | Reference | 29/15 | Reference | 59/43 | Reference |
| | WM+MM | 17/13 | 0.80 (0.31−2.10) | 32/32 | 0.42 (0.16−1.08) | 3/3 | 0.57 (0.09−3.53) |
| | | | 0.654 | | 0.071 | | 0.541 |
| *Ever* | WW | 53/38 | Reference | 41/35 | Reference | 72/89 | Reference |
| | WM+MM | 20/41 | 0.63 (0.31−1.29) | 34/63 | **0.42 (0.21−0.84)** | 2/10 | 0.30 (0.06−1.60) |
| | | | 0.210 | | **0.013** | | 0.160 |

COX-2 indicates the cyclooxygenase-2 gene; OR, odds ratio; CI, confidence interval; WW, homozygous wild-type genotype; WM, heterozygous genotype; MM, homozygous variant genotype.

* Adjusted for age, gender, ethnicity, smoking status, and pack-years for the overall analysis; adjusted for age, sex, ethnicity, and pack-years for the analysis that was stratified by smoking status; adjusted for age, sex, ethnicity, and smoking status for the analysis that was stratified by smoking level; adjusted for age, ethnicity, smoking status, and pack-years for the analysis that was stratified by sex; adjusted for sex, ethnicity, smoking status, and pack-years for the analysis that was stratified by age.

** WW: homozygous wild-type genotype; WM: heterozygous genotype; MM: homozygous variant genotype.

**Table 2**

Association of *COX-2* Haplotypes* and OPL Risk Stratified by Patient Characteristics

| Variables | H1 (WWW**) | | H2 (WMW) | | H3 (MMW) | | H4 (MMM) | |
|---|---|---|---|---|---|---|---|---|
| | Cases/Controls | OR (95% CI)# | Cases/Controls | OR (95% CI) | Cases/Controls | OR (95% CI) | Cases/Controls | OR (95% CI) |
| | | *P* | | *P* | | *P* | | *P* |
| **Overall** | | | | | | | | |
| | 173/191 | Reference | 53/37 | **0.55 (0.33−0.93)** | 47/32 | 0.62 (0.35−1.08) | 7/3 | 0.38 (0.09−1.60) |
| | | | | **0.027** | | 0.093 | | 0.187 |
| **Age** | | | | | | | | |
| *Young* | 80/97 | Reference | 23/17 | 0.49 (0.22−1.09) | 26/14 | **0.33 (0.13−0.83)** | 4/2 | 0.25 (0.04−1.47) |
| | | | | 0.080 | | **0.019** | | 0.126 |
| *Old* | 93/94 | Reference | 30/20 | 0.65 (0.31−1.35) | 21/18 | 0.80 (0.36−1.80) | 3/1 | 0.49 (0.05−5.15) |
| | | | | 0.247 | | 0.590 | | 0.555 |
| **Gender** | | | | | | | | |
| *Male* | 90/106 | Reference | 29/26 | 0.58 (0.28−1.21) | 32/17 | **0.44 (0.20−0.96)** | 3/3 | 0.55 (0.08−4.04) |
| | | | | 0.145 | | **0.040** | | 0.58 |
| *Female* | 83/85 | Reference | 24/11 | **0.42 (0.18−0.99)** | 15/15 | 0.84 (0.36−1.96) | 4/0 | N/A |
| | | | | **0.047** | | 0.681 | | |
| **Smoking status** | | | | | | | | |
| *Never* | 92/58 | Reference | 31/10 | 0.53 (0.24−1.15) | 24/16 | 1.07 (0.47−2.41) | 6/0 | N/A |
| | | | | 0.108 | | 0.874 | | |
| *Ever* | 81/133 | Reference | 22/27 | 0.60 (0.29−1.24) | 22/16 | **0.37 (0.17−0.81)** | 1/3 | 1.16 (0.10−13.88 ) |
| | | | | 0.168 | | **0.013** | | 0.909 |
| **Alcohol status** | | | | | | | | |
| *Never* | 56/85 | Reference | 21/18 | 0.39 (0.15−1.04) | 12/13 | 0.57 (0.20−1.63) | 2/3 | 0.54 (0.09−3.13) |
| | | | | 0.059 | | 0.294 | | 0.493 |
| *Ever* | 117/106 | Reference | 32/19 | 0.55 (0.28−1.09) | 35/19 | 0.60 (0.29−1.22) | 5/0 | N/A |
| | | | | 0.086 | | 0.160 | | |

OR, odds ratio; CI, confidence interval; WW, homozygous wild-type genotype; WM, heterozygous genotype; MM, homozygous variant genotype.

N/A: Not available.

*Order of SNPs comprising *COX-2* haplotype: −765G>C, exon 10 +837T>C, and exon 10 −90C>T.

** W: wild-type allele, M: variant allele.

# Adjusted for age, gender, ethnicity, smoking status, and pack-years for the overall analysis; adjusted for age, sex, ethnicity, and pack-years for the analysis that was stratified by smoking status; adjusted for age, sex, ethnicity, and smoking status for the analysis that was stratified by smoking level; adjusted for age, ethnicity, smoking status, and pack-years for the analysis that was stratified by sex; and adjusted for sex, ethnicity, smoking status, and pack-years for the analysis that was stratified by age.

## Table 3

Associations of *COX-2* Diplotypes with OPL Risk

| Diplotype | Controls (%) | Cases (%) | Adjusted OR (95% CI)* | *P* |
|---|---|---|---|---|
| **Overall** | | | | |
| WWW / WWW | 49 (34.27) | 69 (52.27) | 1.00 (ref.) | |
| WWW / WMW | 33 (23.08) | 26 (19.70) | **0.44 (0.22−0.89)** | **0.022** |
| WWW / MMW | 34 (23.78) | 23 (17.42) | 0.51 (0.26−1.02) | 0.055 |
| WMW / WMW | 6 (4.20) | 2 (1.52) | 0.29 (0.50−1.62) | 0.157 |
| WMW / MMW | 8 (5.59) | 7 (5.30) | 0.63 (0.20−2.00) | 0.429 |

OR indicates odds ratio; CI, confidence interval; W, wild-type allele; WW, homozygous wild-type genotype; WM, heterozygous genotype; M, variant allele; MM, homozygous variant genotype.

*Adjusted for age, gender, ethnicity, smoking status, and alcohol consumption.

**Table 4**

Joint Effects between Genotypes and Smoking Status for OPLs

| Genotype | Exposure Status | Controls (%) | Cases (%) | Adjusted OR (95% CI)* | $P_{interaction}$ |
|---|---|---|---|---|---|
| **Smoking status** | | | | | |
| ***COX-2* −765G>C, rs20417** | | | | | |
| WM+MM | No | 28 (19.44) | 17 (12.69) | 1.00 (ref.) | |
| WM+MM | Yes | 26 (18.06) | 20 (14.93) | 1.29 (0.55−3.06) | |
| WW | No | 51 (35.42) | 26 (19.40) | 0.77 (0.35−1.68) | |
| WW | Yes | 39 (27.08) | 71 (52.99) | 2.99 (1.43−6.23) | **0.041** |
| ***COX-2* exon 10 +837T>C, rs5275** | | | | | |
| WM+MM | No | 54 (37.24) | 25 (18.38) | 1.00 (ref.) | |
| WM+MM | Yes | 41(28.28) | 41 (30.15) | 2.22 (1.15−4.29) | |
| WW | No | 26 (17.93) | 19 (13.97) | 1.53 (0.71−3.31) | |
| WW | Yes | 24 (16.55) | 51 (37.50) | 4.95 (2.48−9.91) | 0.465 |
| ***COX-2* exon 10 −90C>T, rs68479** | | | | | |
| WM+MM | No | 10 (6.90) | 1 (0.74) | 1.00 (ref.) | |
| WM+MM | Yes | 3 (2.07) | 4 (2.94) | 14.73 (1.12−192.90) | |
| WW | No | 70 (48.28) | 43 (31.62) | 6.24 (0.76−51.33) | |
| WW | Yes | 62 (42.76) | 88 (64.71) | 15.60 (1.91−127.63) | 0.184 |

OR indicates odds ratio; CI, confidence interval; COX-2, cyclooxygenase-2 gene; rs, reference single-nucleotide polymorphism; WM, heterozygous genotype; MM, homozygous variant genotype; WW, homozygous wild-type genotype.

*Adjusted for gender and age.